ON THE PROVISIONAL ARRANGEMENT OF THE EMBRYONIC LYMPHATIC SYSTEM

AN ARRANGEMENT BY MEANS OF WHICH A CENTRIPETAL LYMPH FLOW TOWARD THE VENOUS CIRCULATION IS CONTROLLED AND REGULATED IN AN ORDERLY AND UNIFORM MANNER, FROM THE TIME LYMPH BEGINS TO COLLECT IN THE INTERCELLULAR SPACES, UNTIL IT IS FORWARDED TO THE VENOUS CIRCULATION

CHARLES F. W. McCLURE

From the Department of Comparative Anatomy, Princeton University

SIX FIGURES

One of the most interesting problems of the lymphatic system is the determination of the manner in which the continuous centripetal lymph flow is established in the embryo, in relation to the developing lymphatic vessels by which it is subsequently conveyed to the venous circulation.

It is well known that the anlagen of the lymphatic system do not normally make their appearance in the embryo until after the haemal vessels have been established. As soon, however, as the haemal vessels begin to function, lymph begins to collect in the intercellular spaces of the embryo and, as we know, is subsequently collected by a set of newly-formed vessels, the lymphatics, which convey it to the venous circulation.

Those who maintain that the lymphatics sprout centrifugally and continuously from the veins, would necessarily hold that the lymph in the intercellular spaces patiently awaits the arrival of closed and hollow outgrowths from the veins, the lymphatics, before it can be received into any portion of the lymphatic system. Such continuous outgrowths from the veins would necessarily take place in a centrifugal direction which is opposed to that of the centripetal flow of lymph they would receive.

It has always proved a difficult matter for some of us to reconcile the view that the direction of the growth of the vessels

THE ANATOMICAL RECORD, VOL. 9, NO. 4
APRIL, 1915

in which the flow takes place should be opposed to that of the flow. One might expect a continuous centripetal lymph flow toward the venous circulation to be established in a gradual manner in the embryo, and to be regulated from the time lymph first made its appearance in the intercellular spaces, until it was continued on to the venous circulation. In fact, one might expect to find some provisional condition of the embryonic lymphatic system which should exactly accord with the maintenance and regulation of such a centripetal flow. *Such a provisional condition of the embryonic lymphatic system I believe I have been able to demonstrate in a positive manner in the living embryo of the trout.*

One of the most salient features noticeable in the development of the lymphatic system of the trout, as well as in that of mammals, is that the main lymphatic channels are formed through a gradual concrescence of independent and discontinuous anlagen or lymph vesicles. These independent lymph vesicles make their appearance in the embryo in a progressive manner along the lines subsequently followed by continuous lymphatic vessels, and, in certain districts of the mammalian embryo, they utilize the static line vacated by degenerating veins, so that certain lymphatic vessels of the body subsequently follow the course of abandoned veins. These independent lymph vesicles of the embryo first become concrescent to form continuous channels contiguous to the points at which the lymphatics establish typical communications with the veins. With these points of lymphatico-venous entry, the vesicles continue to become concrescent in a progressive manner, so that the outlying or peripheral lymph vesicles are the last to establish a communication with the veins.

The view which calls for the development of the main lymphatic channels through a confluence of independent and discontinuous anlagen was advanced by Huntington and McClure[1]

[1] Huntington and McClure. The development of the main lymph channels of the cat in their relations to the venous system. Anat. Rec., vol. 1, 1907. Read before the American Association of Anatomists at the meeting held in New York in December, 1906.

in 1906. Since a recognition of this fact is a necessary corollary to the main issue involved in the present paper, we will first consider what constitute the main lymphatic channels in the embryo of the trout and show how, in their development, they follow this plan.

Figure 1 represents a ventral view of a reconstruction of the main lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo, on the twenty-second day after fertilization. This embryo was developed at a temperature of about 10.5° C. and its lymphatic system is represented, for the most part, by a continuous system of vessels which drain into the veins at typical points. The typical points of lymphatico-venous communication in the regions of the head and pharynx occur in the cardino-Cuvierian district (*9*); with the precardinal (jugular) vein near the caudal end of the otocyst (*13*); and with the precardinal, near a point where the latter leaves the cranial cavity (*2*). The first and last mentioned points of communication appear invariably to be retained in the adult.

The principal or main lymphatic vessels found in the regions of the head and pharynx of a twenty-two day rainbow trout embryo are as follows:

1. The subocular lymph sacs (saccus lymphaticus subocularis, 1 in figure 1)

The subocular lymph sacs of the trout embryo consist of two relatively huge sacs or vesicles, each of which lies ventro-medial to the eye. They are more or less triangular in form, with their apices directed forward. In the twenty-two day trout under consideration, they extend between the hyoidean artery (*15*) and the olfactory invagination (*21*), as shown in figure 2. At its postero-lateral angle, each subocular lymph sac (*1*) communicates directly with the lateral pharyngeal lymphatic (*3* in fig. 1) and it is solely through the latter vessel that the subocular lymph sac drains into the veins.

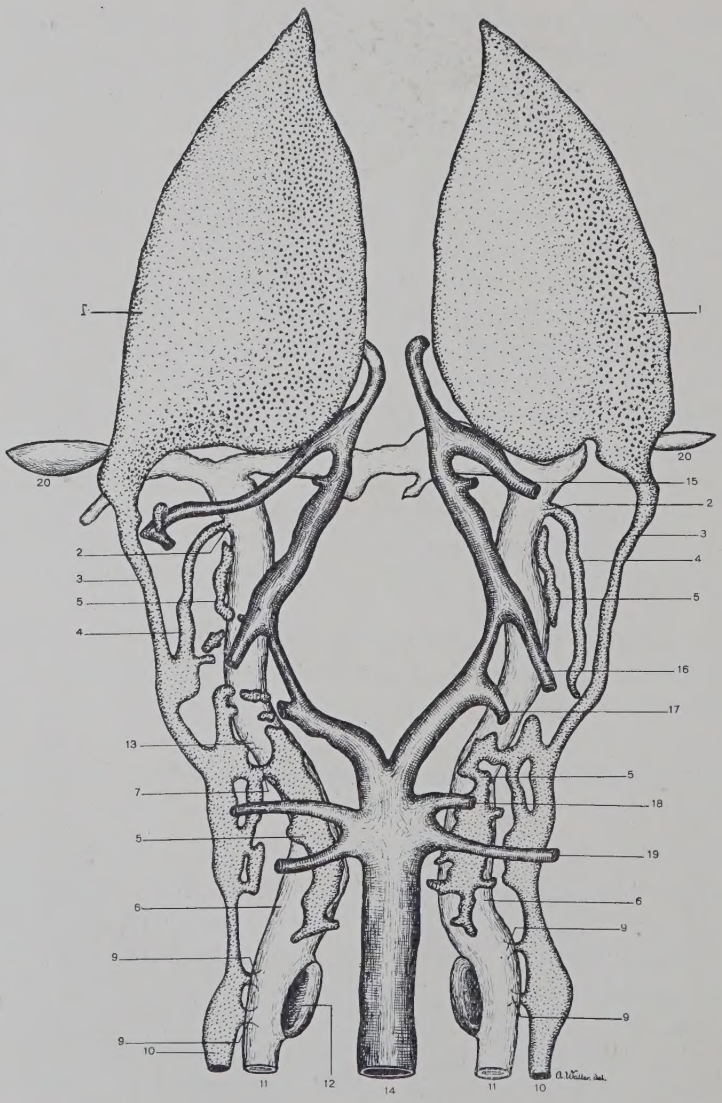


Fig. 1 Reconstruction of the main lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-two-day rainbow trout embryo; ventral view. P. E. C. series 648. Reconstructed after the method of Born at a magnification of 200 diameters.

REFERENCE NUMBERS

1, Subocular lymph sac
2, Medial pharyngeal communication
3, Lateral pharyngeal lymphatic
4, Medial pharyngeal lymphatic
5, Precardinal (jugular) lymphatic
6, Precardinal (jugular) vein
7, Communication between the precardinal lymphatic and the lateral pharyngeal lymphatic
8, Caudal end of otocyst
9, Cardino-Cuvierian communication
10, Lymphatic of the lateral line of the trunk
11, Postcardinal vein
12, Duct of Cuvier
13, Otic communication
14, Dorsal aorta
15, Hyoidean artery
16, First efferent aortic arch
17, Second efferent aortic arch
18, Third efferent aortic arch
19, Fourth efferent aortic arch
20, Caudal end of eye
21, Olfactory invagination
22, Carotid artery

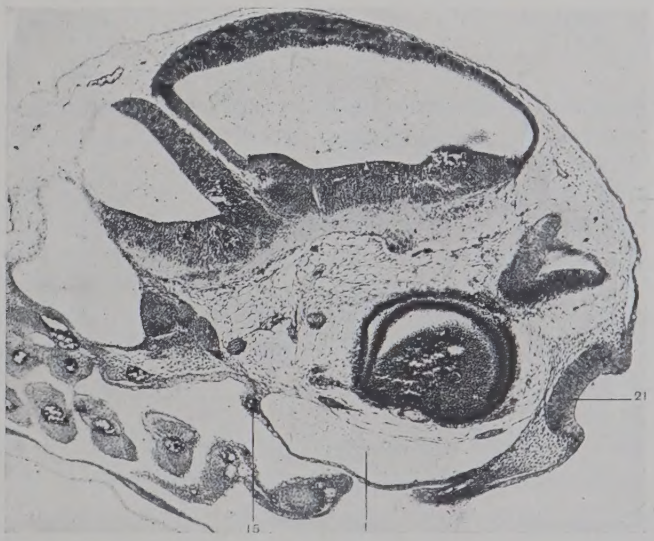


Fig. 2 Sagittal section taken through the head and pharynx of a twenty-two-day rainbow trout embryo, showing the relations of the subocular lymph sac to the eye, the hyoidean artery and the olfactory invagination; *1*, subocular lymph sac; *15*, hyoidean artery; *21*, olfactory invagination. P. E. C. series 816.

2. The lateral pharyngeal lymphatic (truncus lymphaticus pharyngeus lateralis, 3 in figure 1)

This vessel occupies a superficial position in the lateral wall of the pharynx and forms, on each side of the body, the direct anterior continuation of the lymphatic of the lateral line of the trunk (truncus lymphaticus longitudinalis lateralis, *10* in fig. 1). The lymphatic of the lateral line of the trunk is completely developed in the twenty-two day rainbow trout and can be followed caudad to the region of the caudal lymph heart. The lateral pharyngeal lymphatic drains the subocular lymph sac and, at a slightly later stage of development, also the dorsal region of the head and pharynx, the operculum and the lower jaw. It communicates with the veins in the cardino-Cuvierian district (*9*) in common with the lymphatic of the lateral line of the trunk (*10*).

3. The medial pharyngeal lymphatic (truncus lymphaticus pharyngeus medialis, 4 in figure 1)

This vessel occupies a more central position and is more deeply situated than the lateral pharyngeal lymphatic. It follows an oblique course, in a postero-anterior direction, from about the middle of the lateral pharyngeal lymphatic with which it subsequently communicates, to open into the precardinal vein just caudal to the point where this vein emerges from the cranial cavity (*2* in fig. 1).

4. The precardinal or jugular lymphatics (truncus lymphaticus precardinalis vel jugularis, 5 in figure 1)

These vessels develop along the line of the precardinal veins. They are not completely established on the twenty-second day in the form of continuous channels and are represented by such vessels only near the caudal end of the pharynx, where they communicate with the lateral pharyngeal lymphatic (*7* in fig. 1).

All of the continuous lymphatic channels described above, as occurring on the twenty-second day in the embryo of the rain-

bow trout, can be readily injected from the veins, through any one of the typical points of communication which are established between the lymphatics and the veins. By injecting into the subocular lymph sacs it is also possible to fill the continuous lymphatic vessels with injecta, as well as the veins. At this particular stage of development, in the absence of lymphatico-venous valves, blood may flow freely into the lymphatics from the veins, at the typical points at which the lymphatics communicate with the veins. In rainbow trout embryos slightly older than twenty-two days, and in which lymphatico-venous valves have been formed, the application of chloretone apparently vitiates the normal function of these valves. Blood may then also flow freely from the veins into the lymphatics and fill up completely all of the continuous lymphatic channels, including the subocular lymph sacs. If such embryos are removed to water in which no chloretone is present, the blood will flow back from the lymphatics into the veins.

The age of the rainbow, steelhead or brook trout embryo in which a continuous system of lymphatic channels, as shown in figure 1, is met with for the first time, naturally varies with the temperature of the water in which development takes place. Much variation is also met with in the rate at which the lymphatic system of the trout develops in different embryos of the same age, as well as upon opposite sides of the same embryo. When developed at a temperature of about 10.5° C., a continuous lymphatic system, as shown in figure 1, is usually found in rainbow and steelhead trout embryos on the twenty-second day after fertilization, which is six or seven days after its earliest anlagen can first be observed in sections.

In all stages of development, prior to the establishment of such a continuous system of lymphatics as shown in figure 1, a condition is invariably met with in the embryo in which the lymphatic system is represented by a progressively appearing series of discontinuous anlagen, that present varying degrees of concrescence with one another to form continuous lymphatic vessels which communicate with the veins, only at the typical points at which the lymphatics establish communications with the

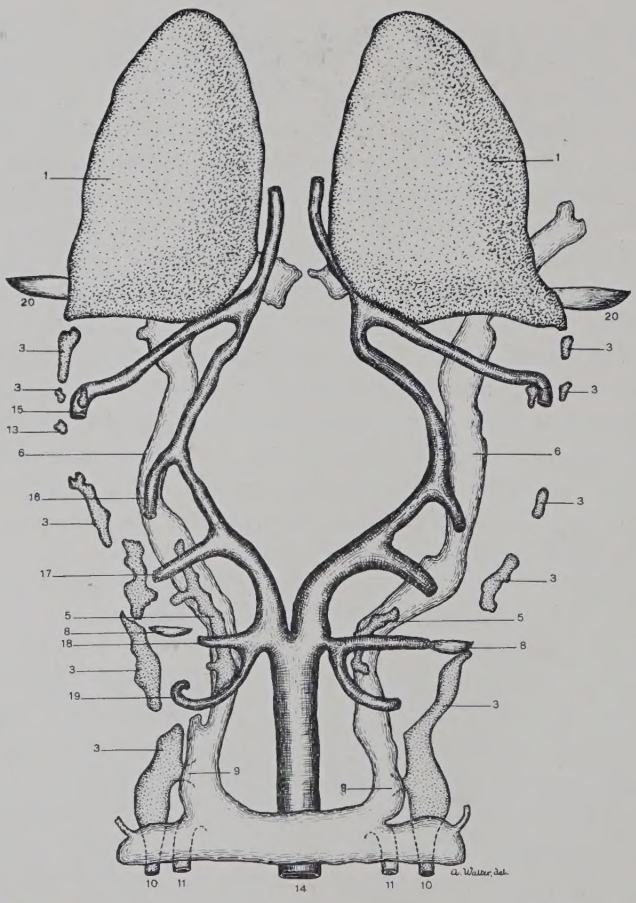


Fig. 3 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-day rainbow trout embryo; ventral view. P. E. C. series 668. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

veins. Two such stages of development are herewith presented in illustration of this fact.

Figures 3 and 4 represent, respectively, reconstructions of the lymphatics, veins, and arteries found in the regions of the head and pharynx of a rainbow trout embryo on the twentieth and

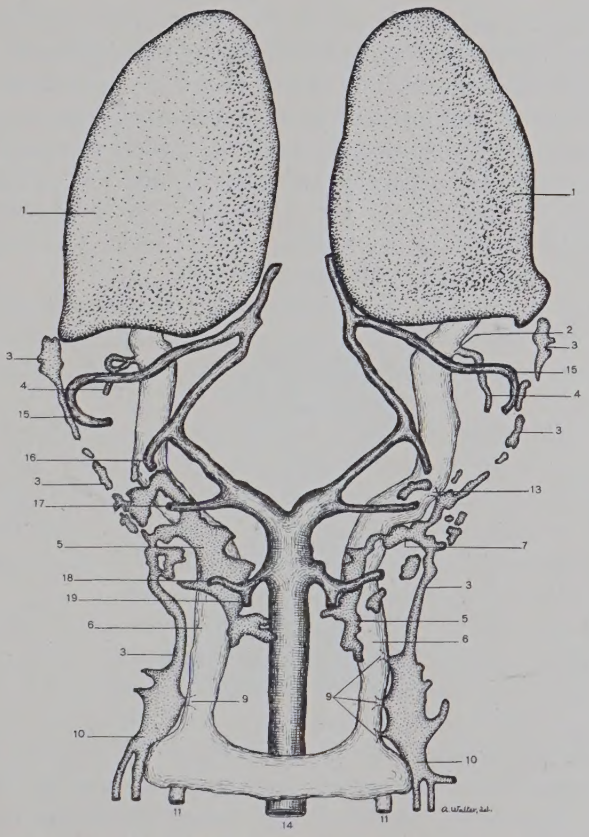


Fig. 4 Reconstruction of the lymphatics, arteries and veins found in the regions of the head and pharynx of a twenty-one-day rainbow trout embryo; ventral view. P. E. C. series 646. Reconstructed after the method of Born at a magnification of 200 diameters; reconstruction of an injected embryo. For reference to numbers see under figure 1.

twenty-first days after fertilization. These embryos were developed at a temperature of about 10.5°C. When compared with the conditions found in the embryo on the twenty-second day (fig. 1), it is seen that the subocular lymph sacs (*1* in figs. 3 and 4) are entirely independent of the veins and of other independent lymph vesicles, and that a series of discontinuous

and independent lymph vesicles lie exactly in the line subsequently followed by continuous lymphatic channels on the twenty-second day (fig. 1). It is also seen that these lymph vesicles present varying degrees of concrescence beginning at the points at which the lymphatics establish typical communications with the veins. It may also be observed that none of these independent lymph vesicles communicate with the veins except those which lie contiguous to the points at which the lymphatics establish typical communications with the veins.

For the purpose of the present paper reconstructions of the twenty and twenty-one day trout are sufficient to illustrate the general principle of development followed by the main lymphatic channels.

The main question involved in the present issue is, however, what functional significance does the presence in the embryo of such an independent and discontinuous series of lymph vesicles imply? *A study of one of these lymph vesicles, the subocular lymph sacs, as observed and experimented upon in the living trout embryo gives, I believe, a positive answer to this question.*

When rainbow and steelhead trout embryos are developed at a temperature of about 10.5°C., the development of the anlagen of the subocular lymph sacs is initiated on about the sixteenth day after fertilization. These anlagen first appear as small clear vesicles which lie in the mesenchyme in an area between the hyoidean artery and the eye and just dorsal to the maxillary ridge. These small vesicles finally become confluent to form a larger vesicle which gradually increases in size. Injection experiments upon the living trout embryo, controlled by sections, have shown that the anlagen of the subocular lymph sacs never establish a communication with the veins in the neighborhood of the sacs. They have also shown that the subocular lymph sacs cannot be injected from the veins at any stage of their development, until after the independent and discontinuous anlagen of the lateral pharyngeal have become concrescent with one another to form a continuous vessel and until a communication has been established between this vessel and the subocular

lymph sac (compare figures 3 and 4 with figure 1). In other words, at no stage of development has it been possible to inject the subocular lymph sac of the trout embryo except by way of the lateral pharyngeal lymphatic, which signifies that the subocular lymph sac of the trout embryo is entirely independent of the veins and of other lymphatics, until this communication has been made.

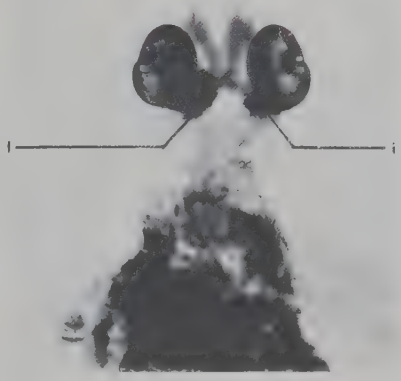


Fig. 5 Photograph of the ventral aspect of the head of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs. Spaltcholz preparation. *1*, subocular lymph sacs.

Injection experiments have also proved that the subocular lymph sacs of the trout embryo do not grow caudad and that they are invariably bounded posteriorly by the hyoidean artery; (compare *15*, hyoidean artery, with *1*, subocular lymph sac, in figures 2, 3 and 4). Figure 5 is a photograph of a Spaltcholz preparation of a twenty-day rainbow trout embryo on which an attempt was made to inject the lymphatics and the veins by injecting into the subocular lymph sacs (*1* in fig. 5). The figure shows the position occupied by the subocular lymph sacs

in the living embryo and, since the sacs alone filled with the injecta, is illustrative of an experiment which proves that the subocular lymph sacs have not grown caudad, nor do they communicate with the lateral pharyngeal lymphatic, nor with the veins at the stage of development presented by this twenty-day trout.

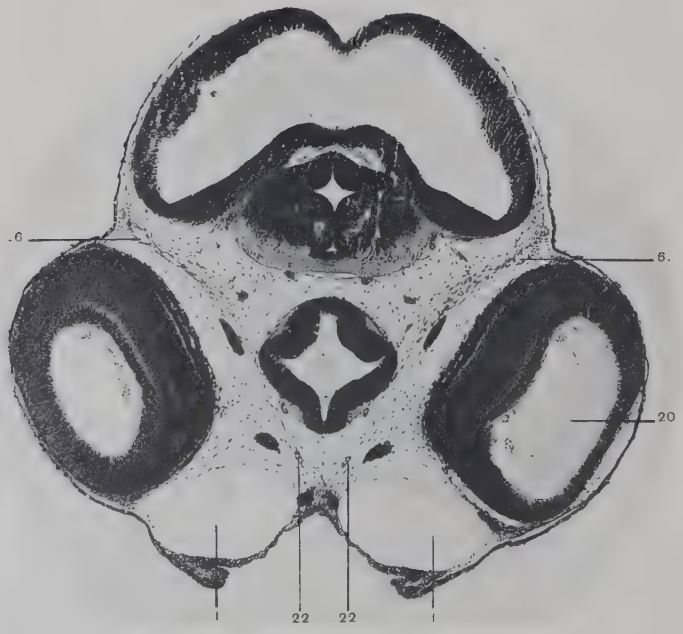


Fig. 6 Transverse section taken through the subocular lymph sacs of a twenty-one-day rainbow trout embryo in which, as independent structures, the sacs have reached the maximum stage of their development. P. E. C. series 646. Injected embryo. *1*, subocular lymph sac; *6*, precardinal (jugular) vein; *20*, eye; *22*, carotid artery.

The subocular lymph sacs of the trout embryo are non-pulsatile in character and are lined by an endothelium around which no muscular coat is formed. During the stage of their independence they become gradually distended with lymph which must necessarily enter them in a centripetal direction from the

intercellular spaces of the head. As this lymph gradually increases in amount the subocular sacs of the trout can be easily observed in the living embryo, and are especially prominent in the rainbow trout between the nineteenth and twenty-first days. By the time the subocular lymph sacs have attained their maximum size as independent structures, on the twenty-first day (figs. 4 and 6), a considerable pressure must be exerted by their lymph upon their walls, which would account for the distended appearance presented by the sacs (*l*) at this stage (fig. 6). As soon, however, as the subocular lymph sacs establish their communication with the lateral pharyngeal lymphatic, so that their lymph can flow continuously and centripetally to the veins, this pressure against their walls is immediately released, and the sacs then appear less prominently in the living embryo, due to a partial collapse of their walls.

It is thus seen that, during the period of its independence, the subocular lymph sac of the trout embryo serves as a local and independent reservoir for the reception of lymph which enters it in a centripetal direction from the intercellular spaces; that it retains this lymph only temporarily, until the sac establishes a communication with the lateral pharyngeal lymphatic, through which a continuous centripetal lymph flow may then pass from the intercellular spaces to the venous circulation.

The subocular lymph sacs of the trout embryo therefore furnish us with a striking example of the fact that, not only do independent and discontinuous anlagen of the lymphatic system actually exist, but, that they can also be observed and be experimented upon in the living embryo.

The functional rôle played by the subocular lymph sacs of the trout embryo, during the stage of their independence, as well as after they have established a communication with the venous circulation, undoubtedly gives us the clue to the function assumed by the independent lymph vesicles of the embryo in general. It also explains the manner in which a continuous centripetal lymph flow is established in the embryo, between the intercellular spaces and the venous circulation, in relation to the developing lymphatic vessels.

On the basis of the functional rôle played by the subocular lymph sacs—and this can be actually demonstrated in the living trout embryo —it is highly probable that the independent lymph vesicles, of the embryo in general, also serve as local reservoirs for the temporary retention of lymph which enters them in a centripetal direction from the intercellular spaces; that these lymph vesicles become progressively concrescent with one another to form continuous channels, through which the lymph collected and temporarily retained by them is then forwarded to the venous circulation. In this manner the centripetal flow of lymph which continuously enters these independent lymph vesicles from the outlying intercellular spaces, is continued on to the venous circulation.

It may be mentioned here, incidentally, that the functional rôle played by the subocular lymph sacs of the trout embryo during the stage of their independence, is also evidence of the fact that the lymphatics of fishes function solely in the capacity of lymphatics at the time of their inception, and that they are therefore not transformed veins.

In case the independent lymph vesicles of the embryo should fail to become concrescent with one another and with the veins, at the typical points of lymphatico-venous entry, an oedematous condition of the body would undoubtedly arise, and the ontogenetic condition, in which only independent and discontinuous anlagen are present, might then be retained in the adult. That such might actually be the case seems to be borne out by the conditions observed in an oedematous human foetus already described by Smith and Birmingham.[2] These investigators have described a case in which that peculiar and rare condition known as oedematous foetus was found to depend upon the complete absence of the thoracic duct, lymphatic glands and lymphatic trunks in general, and in which the lymph was stored in what they described as "greatly distended tissue spaces" which neither communicated with one another, nor with the veins.

[2] Smith and Birmingham. Absent thoracic duct causing oedema of a foetus. Jour. Anat. and Physiol., vol. 23.

Only two possible conclusions can be drawn regarding the significance of the complete absence of continuous lymphatic trunks and of the presence only of independent and discontinuous lymph-containing tissue spaces in this human foetus: Either there has been a complete failure on the part of the lymphatic system even to initiate its development, so that these 'tissue spaces' are in no sense related to the true lymphatic system, or, the presence of these spaces signifies a condition in which the normal development of the lymphatic system has been arrested at an early ontogenetic stage.

Huntington and the writer[3] have repeatedly described and figured the presence of independent lymph vesicles or lymph spaces in the mammalian embryo, and Huntington[4] has recently made a more extensive and detailed study of these structures, as they occur in the subclavian and primitive ulnar regions of the cat. Although one is not given the opportunity of studying these independent lymph vesicles in the living embryo of the mammal, as is the case in the trout, it would now seem quite a waste of time to parley further over the question of their presence in the mammalian embryo, or, that it is through the concrescence of such independent vesicles that the main lymphatic channels of mammals are formed.

The oedematous human foetus described by Smith and Birmingham appears to present us with the most striking evidence, not only of the fact that the presence of independent lymph vesicles may actually be demonstrated, in certain circumstances, as functional structures in mammals, but, also, that the functional rôle played by them is similar to that played by the subocular lymph sacs of the trout during their independent stage. It is

[3] Huntington and McClure. The anatomy and development of the jugular lymph sacs in the domestic cat (Felis domestica). Amer. Jour. Anat., vol. 10, 1910, fig. 66.

[4] Huntington. The development of the mammalian jugular lymph sac, of the tributary primitive ulnar lymphatic and the thoracic ducts from the viewpoint of recent investigations of lymphatic ontogeny, together with a consideration of the genetic relations of lymphatic and haemal vascular channels in the embryos of amniotes. Amer. Jour. Anat., vol. 16, 1914. Also, The development of the lymphatic drainage of the anterior limb in embryos of the cat. Proc. Amer. Ass. Anat., Anat. Rec., vol. 9, 1915.

highly probable, therefore, that the conditions found in this human foetus indicate that the development of the lymphatic system had been arrested at a normal ontogenetic stage; that its oedematous condition was due to the circumstance that the independent and discontinuous anlagen of the lymphatic system had failed to become concrescent with one another and with the veins, in order to establish a continuous system of channels, through which a continuous centripetal lymph flow could pass from the outlying tissue spaces to the venous circulation.

THE PYRAMIDAL TRACT IN THE GUINEA-PIG (CAVIA APEREA)

IDA L. REVELEY

From the Physiological Laboratory, Medical College, Cornell University, Ithaca, N. Y.

TEN FIGURES

INTRODUCTION

The pyramidal tract (fasciculus cortico-spinalis) in rodents, so far as it has been examined in this order, is crossed and runs in the dorsal column of the spinal cord, but there are exceptions to this rule. In the family Leporidae, including the rabbits and hares, it lies in the lateral columns, and in the Canadian porcupine there is a dorsal column, a lateral column and a ventral column tract (Simpson '14). In view of the fact, therefore, that such wide variation exists between closely related species, it is desirable that as many as possible of these be examined.

In the guinea-pig, the animal with which this paper deals, Spitzka ('86), Bechterew ('90) and Wallenberg ('03) have found that the pyramidal tract decussates into the posterior column.

Ranson ('13) states that in the albino rat the tract consists of a mixture of medullated and non-medullated fibers, and by the use of the pyridine-silver method of Cajal (modified), the non-medullated fibers are stained, so that the course of the tract can be followed by this means.

According to Linowiecki ('14), who worked in Ranson's laboratory, also with the pyridine-silver method, the same obtains in the guinea-pig. In this animal the tract lies in the posterior column, but it does not form such a compact uniform area when stained by this method as is found in the rat, indicating, apparently, that the proportion of medullated to non-medullated fibers is greater in the guinea-pig.

The pyridine-silver method may be regarded as the complement of the Marchi method since the latter stains only medullated fibers in the process of degeneration.

PRESENT INVESTIGATION

The object of the present research was to trace the fibers of the pyramidal tract in the guinea-pig from their origin in the cerebral motor cortex to their termination in the lower levels of the brain and spinal cord. The method of secondary degeneration was employed, with Marchi staining.

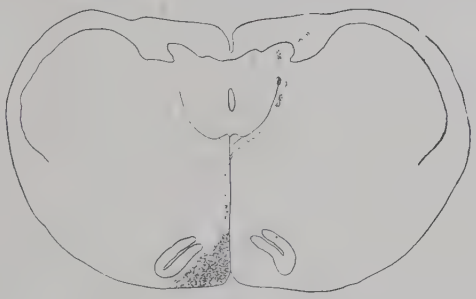
Eight animals (adults) in all were used. The cerebrum was exposed on the left side, under ether anesthesia, and the motor cortex removed. At the end of periods varying from twelve to sixteen days after the operation they were killed by ether or coal gas, when the brain and spinal cord were removed and placed in 3 per cent potassium bichromate. After three weeks in this fluid, with frequent changing, the tissue was cut into slices 3 to 4 mm. thick and placed in Marchi's fluid (3 per cent potassium bichromate, 4 parts, 1 per cent osmic acid, 1 part). At the end of eighteen days the pieces were removed, washed in running tap water for twelve hours, and taken through the alcohol-xylene-paraffin series into paraffin in which they were imbedded and cut. Sections from all levels of the brain and from most of the segments of the spinal cord were mounted and examined.

COURSE OF PYRAMIDAL TRACT FOLLOWED BY SECONDARY DEGENERATION

The course of the pyramidal tract through the midbrain, pons and upper part of medulla oblongata is similar to that found in the higher mammals such as the cat, dog, monkey and man, and is so well known that no detailed description need be given. In the midbrain it occupies the middle three-fifths of the crusta, more or less, and is continued downwards as the pontine bundles, which unite at the lower border of the pons to form the anterior pyramid of the medulla. Above the level of

the general decussation, in the lower part of the medulla oblongata, there is no evidence of any crossing of fibers; all the degeneration appears to be confined to the side of the lesion.

Sections through the lower or closed half of the medulla oblongata, about 1 mm. below (caudal to) the calamus scriptorius, show the beginning of the pyramidal decussation. The



1

2

Fig. 1 Transverse section, medulla oblongata through upper extremity of pyramidal decussation. × 10.

Fig. 2 Transverse section, medulla oblongata through middle of pyramidal decussation. × 10.

pyramid, in transverse section, is triangular in outline at this level, and from the dorso-mesial angle a few fibers can be seen passing backwards along the median raphé. They cross the raphé close to the central gray matter and curving outwards, in front of the hypoglossal nucleus, turn backwards in the gray substance. One or two small bundles reach the posterior column but most disappear in the gray matter (fig. 1).

In sections at a lower level, about the middle of the decussation, the fibers cross in great numbers and in more or less well defined bundles which follow an undulating course, interlacing with corresponding bundles from the sound side. After crossing the raphé the fibers turn outwards and then curve backwards and inwards through the gray matter, most of them passing into the funiculus cuneatus, where, cut transversely, they form a distinct and compact tract (fig. 2). Along the dorsal margin of the gray matter a few small bundles are seen on the mesial side of the main crossed tract.

At this level a single small strand of degenerated fibers runs backwards through the gray matter on the same side, close to the central canal, and then makes a sharp bend outwards; it disappears in the gray matter before it reaches the dorsal column. It is a very small bundle, with a horizontal course, since it is present only in four consecutive sections.

At the junction of the medulla with the spinal cord (fig. 3) practically all the fibers have crossed and the tract formed lies in the funiculus cuneatus. It is more or less triangular in outline, but a few small detached bundles extend from its mesial angle along the dorsal margin of the gray matter, as described in the last section. The homolateral bundle, seen near the middle of the decussation, is absent at this level; the crossing seems to be complete, no degeneration being visible on the same side. Between the upper and lower limits of the decussation many fibers seem to have disappeared since the degeneration in the anterior pyramid is denser and occupies a more extensive area than in the crossed dorsal column tract. These have presumably terminated in the gray matter of the bulb in this region.

In the first cervical segment the crossed pyramidal tract reaches its largest size. It lies in the column of Burdach of the opposite side, in contact with the posterior horn and gray commissure. It is somewhat triangular in outline, its ventromesial angle extending to the middle line and meeting its fellow of the homolateral side (fig. 4). All the fibers of the tract have decussated and there is no evidence of any degeneration in the

crossed lateral or direct ventral columns as is the case in the Canadian porcupine.

Sections through the second cervical segment (fig. 5) show a considerable change in the area occupied by the fibers of the tract. It is crescent-shaped; the dorsal border is concave; the mesial border lies against the posterior medium septum, oc-

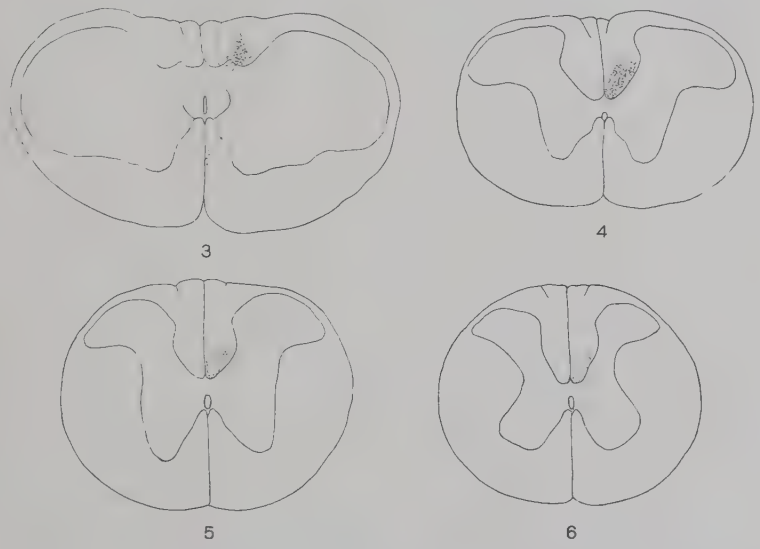


Fig. 3 Transverse section, medulla oblongata through lower (caudal) extremity of pyramidal decussation. × 10.

Fig. 4 Transverse section, first cervical segment of spinal cord. × 10.

Fig. 5 Transverse section, second cervical segment. × 10.

Fig. 6 Transverse section, fifth cervical segment. × 10.

cupying about one-fourth of the distance between the posterior gray commissure and the free margin of the section. The degeneration is less dense than in the first cervical segment indicating a distinct diminution in the number of fibers.

In the third, fourth and fifth cervical segments (fig. 6) the general appearance of the tract changes little, but there is a progressive falling off in the number of fibers which it contains.

The degeneration seems to be densest near the gray matter, the fibers becoming more and more scattered towards the dorsal border of the area.

Between the fifth cervical and first thoracic segments (fig. 7) a still further diminution in the number of fibers is evident. In the latter segment the tract, considerably reduced in size, occupies an oval area which is no longer in contact with the posterior median septum except at its ventro-mesial extremity.

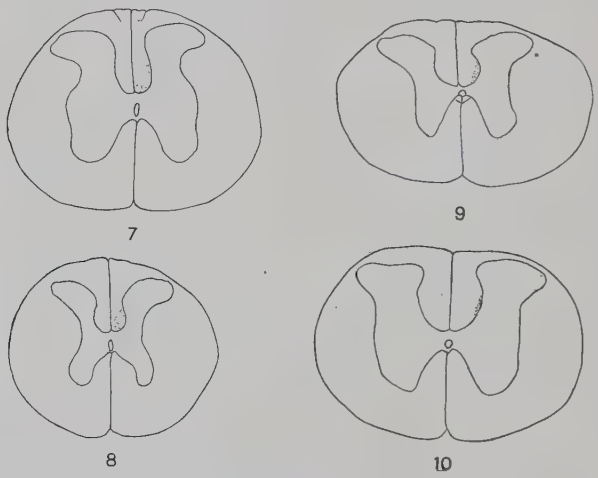


Fig. 7 Transverse section, first thoracic segment. × 10.
Fig. 8 Transverse section, eighth thoracic segment. × 10.
Fig. 9 Transverse section, first lumbar segment. × 10.
Fig. 10 Transverse section, fourth lumbar segment. × 10.

In the eighth thoracic segment the area of degeneration is still more restricted (fig. 8). It has now withdrawn from the middle line and lies in the recess formed by the narrowing of the neck of the posterior horn. Tracing it caudalwards it is found to occupy the same relative position in the succeeding segments, becoming more and more reduced in size until the fourth lumbar segment is reached, where it is represented by a very small number of scattered fibers lying against the neck of the posterior horn (figs. 9–10). Beyond this level it cannot be followed.

It is interesting to compare the above results, obtained by the Marchi method, where the medullated fibers alone are stained, with those of Linowiecki, in the same animal (guinea-pig), who used the pyridine-silver method which brings out the non-medullated fibers. According to his description: "In the seventh cervical segment the pyramidal tract is located in the ventral part of the posterior funiculus. . . . . The fibers of the tract are more densely grouped ventrally and laterally near the grey substance and this gives the cross section of the two tracts somewhat the form of the letter V." (Compare with figures 6 and 7.)

At the level of the eighth thoracic segment, by the pyridine-silver method, the tracts are crescentic in outline and much diminished in size; they are still further reduced at the twelfth thoracic segment where they consist of two compact groups of axons which have become separated at the posterior median septum. Proceeding caudalwards they become less distinct and at the level of the second lumbar segment the groups tend to move posteriorly and to separate from each other. From here on they narrow markedly and fade in color until at the level of the fifth lumbar segment they consist of two narrow strips, one on each side of the posterior median septum, which are hardly visible.

It will thus be seen that the descriptions of the position and outline of the pyramidal tract, as brought out by the two methods, are in close agreement. This would indicate that the mixture of medullated and non-medullated fibers, of which the tract appears to be made up, is more or less uniform throughout its entire course in the spinal cord.

In the fifth lumbar segment, according to Linowiecki, "the tracts consist of two narrow strips on each side of the posterior medium septum,"[1] but he does not say whether they are in contact at the septum or separated from each other. At the level of the fourth lumbar segment almost the same words might

[1] Taken as it stands, this sentence would seem to indicate that the tract is represented by *two* narrow strips on each side. What the author does mean, probably, is that there are two narrow strips, *one* on each side.

be used to describe the tract, as brought out by the degeneration method, if it be added that the narrow strip lies close to the mesial aspect of the gray matter forming the neck of the posterior horn (fig. 10).

SUMMARY

The course of the pyramidal tract in the guinea-pig, from the beginning of the decussation in the medulla oblongata caudalwards, as brought out by the method of secondary degeneration, with Marchi staining, is as follows:

The decussation begins about 1 mm. below the level of the calamus scriptorius and ends near the junction of the medulla with the spinal cord. All the fibers cross, between these limits, and most pass on into the funiculus cuneatus where they turn caudalwards into the spinal cord but many end in the gray matter of the bulb in this region. As this dorsal column tract is followed downwards, from segment to segment of the cord, its outline changes considerably (see figures) and there is a progressive diminution in the number of fibers which it contains, but this loss of fibers is most marked in the upper cervical and lower thoracic regions.

The tract cannot be traced farther than the fourth lumbar segment, where it is represented by a very few degenerated fibers lying close to the gray matter of the posterior horn.

According to Ranson the pyramidal tract consists of a mixture of medullated and non-medullated fibers, the former of which, while undergoing degeneration, may be stained by the Marchi method, the latter by the pyridine-silver method. The description of the spinal portion of the tract in the guinea-pig given by Linowiecki, who used the pyridine-silver method, is in close agreement with what I have found by the degeneration method; this would appear to point to the fact that the mixture of the two varieties of fibers, within the tract, is fairly uniform throughout its course.

BIBLIOGRAPHY

Bechterew, W. 1890 Ueber die verschiedenen Lagen und Dimensionen der Pyramidenbahnen beim Menschen und den Tieren und über das Vorkommen von Fasern in denselben, welche sich durch eine frühere Entwickelung anszeichnen. Neurol. Centrabl., p. 738.

Linowiecki, A. J. 1914 The comparative anatomy of the pyramidal tract. Jour. Comp. Neur., vol. 24, p. 509.

Ranson, S. W. 1913 The fasciculus cerebro-spinalis in the albino rat. Amer. Jour. Anat., vol. 14, p. 411.

Simpson, S. 1914 The motor areas and pyramid tract in the Canadian porcupine (Erethizon dorsatus, Linn.) Quart. Jour. Exper. Physiol., vol. 8, p. 79.

Spitzka, E. C. 1886 The comparative anatomy of the pyramid tract. Jour. Comp. Med., vol. 7, p. 1.

Wallenberg, C. A. 1903 Cited by Goldstein. Zur vergleichenden Anatomie der Pyramidenbahn. Anat. Anz., Bd. 24, p. 454.

A STUDY OF THE AFFERENT FIBERS OF THE BODY WALL AND OF THE HIND LEGS TO THE CEREBELLUM OF THE DOG BY THE METHOD OF DEGENERATION

GILBERT HORRAX

From the Anatomical Laboratory of the Johns Hopkins Medical School

SEVEN FIGURES

This work was begun under the stimulus of the work of Bolk, who based an hypothesis of a localization of a series of coördinating centers in the cerebellum upon studies in comparative anatomy. Bolk has simplified the nomenclature of the parts of the cerebellum and, using his terms, sums up the localization in the cerebellum as follows:

> The lobus anterior cerebelli contains the coördinating centers for the groups of muscles of the head, namely those of the eyes and tongue, the muscles of mastication and muscles of expression beside those of the larynx and pharynx; the lobulus simplex contains the coördinating centers for the neck musculature; the upper part of the lobulus medianus posterior contains the unpaired coördinating centers for the right and left extremities; the lobuli ansiformes and paramediani contain the paired centers for the two extremities, while the rest of the cerebellum has the coördinating centers for the trunk musculature ('07, p. 170).

In general, Bolk thinks that the coördinating centers for symmetrical muscles which act together are in the vermis, while the centers for those which act independently are in the hemispheres.

This hypothesis has been borne out by the experimental work of Rynberk ('04) from Luciani's laboratory, for example, by obtaining special movements of the neck muscles after a unilateral extirpation of the lobulus simplex. These results suggest further work on the end station of the fibers of the different regions of the body by the method of degeneration, though

it is of course clear that the tracing of the afferent fibers of each region to their end station does not unravel the nature of a coördinating center in the cerebellum. The results of tracing the fibers of the different regions of the cord to the cerebellum indicate that the fibers of each of the regions of the body sends fibers to almost the entire vermis.

The most recent and the most extensive work on determining the distribution of spinal fibers in the cerebellum is that of Sir Victor Horsley in 1909. In this article he gives a complete analysis of the literature of the work on the fasciculis spino-cerebellares ventralis and dorsalis. As far as the point of the distribution of the fibers to the cerebellum is concerned, the main results are as follows: In 1890 Auerbach stated that the fasciculus dorsalis (Flechsig) ended in the dorsal—that is to say, in the cephalic—part of the superior vermis, and the fasciculus ventralis (Gower's) in the ventral part. In 1892 Mott reversed this statement by showing that the fasciculus dorsalis ends in the vermis, caudal to the end station of the fasciculus ventralis, which enters the cerebellum farther cerebralwards by way of the brachium conjunctivum or superior cerebellar peduncle. This point is well shown in the well-known diagram of his figure 1, page 219.

Collier and Buzzard in 1903, in an analysis of human material, confirmed Mott's view of the relative position of the end station of the dorsal and ventral cerebellar tracts; that is, that the fasciculus spino-cerebellaris dorsalis ends in the inferior vermis, though they find that some of the fibers end in the nucleus dentatus and in the nuclei of the roof. The fasciculus spino-cerebellaris ventralis they trace by way of the superior cerebellar peduncle to the superior vermis but in small part also to the lateral hemispheres.

Sir Victor Horsley divided the cord in a general way into four regions: the first, from the first to the fourth cervical segment, representing movements of the head and neck; the second, from the fifth cervical to the first thoracic segment, representing movements of the arm; the third, from the second thoracic to the second lumbar, representing movements of the body; and the

fourth, from the third lumbar to the second sacral, representing movements of the leg. He made the lesion cover the fibers representing a given region, by destroying the cells of origin for the tract rather than the fiber tract itself. Indeed, his purpose was to determine which cells of the cervical and lumbar regions of the cord are homologous with the nucleus dorsalis. The lesion for the first or upper cervical region was in the gray matter of the cord, taking in the cells in the homologous position to the nucleus dorsalis and the cells of the middle region of the gray matter. Within the cerebellum the fibers passed to all the vermis except the most anterior part of the lobus anterior, namely, the lingula, and the most posterior part of the lobulus medianus posterior, namely, the uvula and the nodulus. Thus the end station for the afferent fibers of the neck is not limited to the lobulus simplex but includes almost the entire anterior lobe and most of the median posterior lobe as well.

The fibers of the second region, representing fibers from the arm, he found to pass forward mainly on the same side but in part on the opposite side. Within the cord they run both in the dorsal and in the ventral cerebellar tracts. Within the cerebellum they end in the lobulus centralis, the ventral half of the culmen and the ventral half of the pyramidalis; or in Bolk's terminology, in the lobus anterior, in all the lobulus simplex and in most of the lobulus medianus posterior. Horsley did not study the fibers of the third of the body regions, but the fibers from the leg region he found ended in exactly the same parts of the cerebellum as those of the arm region.

Thus from the literature it is clear that the spino-cerebellar fibers end in the vermis; that the fasciculus spino-cerebellaris ventralis (Gower's tract) ends in the more cerebral part of the vermis, while those of the fasciculus spino-cerebellaris dorsalis end in the more caudal part of the vermis. The fibers representing the four regions of the body namely, the neck, the arms, the body and the legs—pass through both cerebellar tracts and are distributed to all parts of the vermis except the most anterior and the most posterior folia. These results are confirmed in the experiments herein reported.

As far as the symptoms of lesions of the cerebellar tracts are concerned, our results also agree with those of Horsley, who found that there was no loss of efferent (purposeful) movement in the muscles involved; that all the motor effects were transitory and probably due to interference with the anterior cornus, and that there was ataxia and clumsiness of movement. These results are practically the same as those of Bing.

TECHNIQUE AND METHOD OF INVESTIGATION

Three dogs, each about the size of an ordinary fox terrier, were used for the purpose of our study, and in all cases the technique of operation and preparation of material was identical. In Dog 1, experiments with regard to various sensations were carried out during the period between the operation and the killing of the animal for histological study. As these experiments were not of a sufficiently satisfactory nature, they were omitted in Dogs 2 and 3, but are recorded in connection with Dog 1 for the sake of completeness. The artificial lesion in the cords of the dogs was made as follows: An incision was made in the median line of the back, extending between the scapulae down toward the lower dorsal region, for a distance of about 10 cm. Very little hemorrhage took place and this was quickly stopped. Laminectomy of the 4th, 5th and 6th dorsal vertebrae was performed and the cord in its dura exposed. An aneurism needle was placed under the cord very gently and the cord in its dura was lifted slightly and rotated a little toward the left. A very superficial slit was then made with a narrow scalpel, through the dura and into the substance of the cord at right angles to its long axis, and on the right side of the animal, in an effort to cut the fasciculus spino-cerebellaris dorsalis (Flechsig) and possibly the fasciculus spino-cerebellaris ventralis (Gowers); the cord was then slipped back. There was no hemorrhage in any case during the cutting of the cord.

The wound was closed in tightly by sutures of silk through the muscles, fascia, subcutaneous tissue and skin. All the dogs made prompt recoveries, but in Dog 2 the skin layer of the

wound was opened by the dog's scratching on his cage. The skin separated, but the wound was kept swabbed out with iodine so that the lower layers did not open and were not involved in the superficial infection. The dog improved steadily and granulations formed rapidly over the wound.

The operations were performed by Dr. Goetsch, of the Johns Hopkins Hospital, on Dog 1; by Dr. Hunnicutt, of the Johns Hopkins Hospital, on Dogs 2 and 3, under strict aseptic precautions, the total time of anesthesia being from an hour and a quarter to two hours; ether was used as an anesthetic.

The dogs were kept alive for periods of ten days to three and one-half weeks, during which time observations were made on them in order to ascertain the nature of the symptoms caused by the lesion. The wounds of the operations healed perfectly within a few days. The symptoms observed were recorded each day, and in brief were as follows:

Dog 1. April 26, 1910, the day after the operation, 9.00 A.M. No apparent disability except in use of hind legs; dog sits at rear of cage, with left leg extended and raised at an angle of 35° to 45°; right hind leg somewhat flexed and lying on floor. When called and coaxed by snapping the fingers, the dog responds by wagging tail, and by slight efforts at movement, but does not actually change position. Head, fore legs and fore feet, are moved in a perfectly coördinated and intelligent way.

April 27, 1910, 9.30 A.M. Dog still sits in about the same position as on previous day, but the left hind leg, instead of being raised, is more nearly or quite touching the floor. When called, the dog crawls to the door of the cage, locomotion being accomplished mainly by the use of the fore legs, the animal remaining in the sitting posture throughout. The hind legs are both more or less flexed, and during locomotion perfectly definite, although almost ineffectual, movements are made by them; the toes of the left hind leg are occasionally flexed. Movements of all parts of body except hind legs seem perfectly normal.

April 28, 1910. When taken out of cage to-day, the dog moves along with its hip against the wall for support, the hind legs not working much better than the day before. Once it sat down and scratched with the right hind leg, just posterior to the right fore leg.

April 29, 1910. When taken out on the grass, the dog took several steps in a normal manner on all four legs, but finally the hind legs weakened, spread apart, and collapsed, throwing the rear half of the dog's body first to one side and then to the other. The dog was seen to scratch again in the same manner as yesterday, only the

left hind leg was used. Hot and cold water was applied to all four legs, by means of dipping the latter into a beaker of water (during these tests the dog was blindfolded). Following are the results:

Temperature of 3° to 0°C. Causes no effect on any of feet.
Temperature of 20° to 37°, 47°, 57° Causes no effect on any of feet.
Temperature of 67° Causes both hind legs to be withdrawn from the water 20 seconds after time of immersion; both front legs were withdrawn 2 to 3 seconds after immersion; pinching toes with forceps causes prompt withdrawal of all four legs.

April 30, 1910. This morning the dog shook the fore part of the body, also got up on all four legs and stood for some time.

Temperature of 37° Gives no effect on any of feet.
Temperature of 47° No effect on hind legs; right front leg was withdrawn when the tips of toes came in contact with the water; left fore leg not tried.
Temperature of 57° No effect on hind legs; right fore leg was withdrawn on being touched to the water; no effect on left fore leg.
Temperature of 58° All legs withdrawn from 6 to 12 seconds after time of immersion, fore legs a little sooner than hind legs; toes of hind legs were shaken in the water before withdrawal.
Temperature of 65° Same as 58°.
Tail withdrawn 6 seconds after immersion in water of 56° temperature.

May 2, 1910. Dog showed marked improvement in walking more normally. Hind legs used most of the time, but with an unsteady, swaying motion from side to side.

Temperature of 37° No effect on hind feet; front feet were withdrawn upon contact with water. Note: When the fore feet were held in the water, no signs of discomfort were apparent.
Temperature of 43° Same as at 37°.
Temperature of 53° Same as at 37°, except that discomfort was evidenced when fore feet were held in the water.
Temperature of 57° Left hind foot withdrawn in 5 seconds; right hind foot withdrawn in 15 seconds; fore feet both withdrawn upon contact.

May 3, 1910. Improved general condition was noted to-day, with better use of hind legs, although the drunken, swaying gait was still marked.

Temperature of 28° No effect on hind feet; fore feet withdrawn upon contact with the water.
Temperature of 35° Hind feet withdrawn after 15 seconds; fore feet withdrawn upon contact.
Temperature of 47° Right hind foot withdrawn upon contact; left hind foot not withdrawn at all; both fore feet withdrawn upon contact; no effect on tail.

May 4, 1910. Temperature observations.

Temperature of 28° Hind feet at first withdrawn upon contact, but subsequently allowed to remain in water; fore feet withdrawn upon contact and not subsequently allowed to remain.
Temperature of 37° No effect on hind feet; fore feet withdrawn upon contact, but allowed to remain upon subsequent immersion.
Temperature of 47° All feet withdrawn almost immediately; when beaker containing no water was touched to the hind feet there was no withdrawal nor other noticeable effect; same beaker to fore foot caused withdrawal of latter; right fore foot was not withdrawn.

May 7, 1910.

Temperature of 42°, No effect on hind feet; fore feet withdrawn upon contact.
Temperature of 45°, 47° Same as 42°.
Temperature of 55° Hind feet withdrawn after 9 seconds; fore feet withdrawn upon contact.

May 10, 1910. Again a marked improvement in the use of the hind legs was noted. Dog was very lively, running and jumping around, but there was still lack of coördination in the hind legs.

May 11, 1910. Dog was livelier than on previous day; ran about and sprang upon the observer in puppy-like fashion, playing around with very little departure from normal movements. However, the same uncertain gait of the hind legs was noticed when the dog would stop jumping and either walked or ran slowly away.

May 13, 1910. Knee jerks of hind legs present and equal on both sides.

May 14, 1910. Dog killed; brain and cord removed.

Dog 2. December 7, 1912. Operation, 10 A.M.
December 7, 1912. Dog conscious and sitting up at 2.30 P.M.
December 8, 1912. Sitting up; can move back legs.
December 9, 1912. Makes attempt at locomotion with hind legs.
December 10, 1912. Can stand up on all fours.

December 11, 1912. Walks a little, with a typical, extreme ataxic gait (back feet sometimes interfering with each other) and often falls to one side or the other.

December 13, 1912. In getting into his box, there seems to be more uncertainty of his right than of his left hind leg; dog walks more to-day, same gait but some improvements.

December 14, 1912. 4 P.M. walks about with considerable ease; follows one around the room, comes when called, etc. His hind legs, however, are very ataxic, the right being noticeably more so than the left.

December 15, 1912. Walks about; shows much improvement in gait.

December 16, 1912. Gait much improved; ataxia in right hind leg still shown.

December 17–20, 1912. Gait steadily improving; also marked gain in weight.

December 20, 1912. Dog killed; brain and cord removed.

Dog 3. December 14, 1912. Operation, 10.30 A.M.

December 14, 1912. 4 P.M.; dog gets up on all fours and walks about the room; his hind legs being very ataxic, but his recovery in general being quicker and his ability to walk coming much sooner than was the case in either Dogs 1 or 2.

December 15, 1912. Dog walks about the room; ataxia of right hind leg.

December 16, 1912. Walks about; ataxia of right hind leg perfectly distinct.

December 17–20, 1912. Improvement rapid and more complete than in Dogs 1 and 2; runs and jumps about the room.

January 8, 1913. By this time no ataxia can be noticed; dog's gait and actions are apparently normal in every way.

January 10, 1913. Dog killed; brain and cord removed.

At periods, varying from 10 days to $3\frac{1}{2}$ weeks from the dates of operation, as noted above, the dogs were anesthetized with chloroform. The right femoral vein was opened, after which a cannula was inserted into the left common carotid artery and through the latter a liter and a half of 10 per cent formalin solution was injected into the animals.

After waiting half an hour in order that as much hardening as possible might take place, the brain and cord were removed carefully. The cord was cut into three approximately equal lengths and put at once into a vessel containing a 10 per cent solution of formalin. The material was left in this solution until various parts of it were wanted for study. The first blocks

were cut out of the cord three days after it had been put into the formalin and the other blocks were taken subsequently for study throughout the following year; all the blocks were from 5 to 10 mm. thick. The part containing the medulla and pons, with the cerebellum, was cut into four blocks which were numbered as is shown in figure 1. As will be seen, the first and second blocks contain, in Bolk's nomenclature, the lobulus medianus posterior of the cerebellum; the third block contains the lobulus simplex and the caudal part of the lobus anterior; the first block contains the rest of the lobus anterior. The blocks were all treated in the same manner; they were stained *en blocke* by a modified Marchi method; they were placed for from 5 to 7 days in the following solution:

Osmic acid	1 part
$NaIO_3$	3 parts
Distilled water	300 parts

The blocks were embedded in celloidin and all the sections showed an excellent staining of the degenerated myelene.

EVIDENCES OF DEGENERATION IN THE SECTIONS

The study of the sections of the first dog showed above the lesion degenerated fibers scattered throughout the section on both sides. In the upper cervical region, as shown in figure 2, there is a very abundant, scattered, bi-lateral degeneration of fibers, covering the entire area of the fasciculus spino-cerebellaris dorsalis and ventralis. It is difficult to understand why there is so extensive a degeneration on the left side, inasmuch as the cord was cut only on the right; but as has been seen, the symptoms involved both sides and there is an almost symmetrical degeneration.

Sections through the first block containing the cerebellum, as shown in figure 3, have a concentration of the degenerated fibers in the corpus restiforme and along the lateral margins of the medulla, with a second concentration in the tractus spino-cerebellaris ventralis, especially of the right side. In the cerebellum the degeneration is confined to a band across the vermis

in its ventral third. This degeneration does not appear until the cephalic end of the first block is approached.

As one follows the sections farther cerebralward in the second block, as seen in figures 4 and 5, there is the same concentration of degenerated fibers in the corpus restiforme and along the right margin, while the fibers on the left side are less numerous but have the same general pattern. Within the cerebellum the degenerated fibers are found throughout the second block, that is, the lobulus medianus posterior. For the most part, the degeneration is confined to the vermis but in figure 5 can be seen a few fibers in the edge of the hemispheres.

From here on the course of the fibers of the fasciculus spino-cerebellaris dorsalis can be followed easily in their position in the inferior cerebellar peduncle. In the third block, as seen in figure 6, the fibers of the corpus restiforme can be seen in the folia of the vermis of the lobulus simplex. The cephalic limit of the ending of the fasciculus spino-cerebellaris dorsalis within the cerebellum is reached in the caudal half of the third block, as shown in figure 6. Above this level, namely, in the lobus anterior, only the fibers of the fasciculus spino-cerebellaris dorsalis are to be found (fig. 7).

It will thus be seen that we have traced those fibers of the fasciculus spino-cerebellaris dorsalis (Flechsig), which represent the legs and possibly the lower body wall, from their situaion in the cord, up through the corpus restiforme of the medulla into the vermis cerebelli, in the caudal half of which they were distributed. In the most caudal part of the vermis their distribution is confined to one or two laminae, but farther cerebralward the distribution is very diffuse throughout all the laminae.

A part of the sensory fibers from the legs and lower body wall pass to the cerebellum through the fasciculus spino-cerebellaris ventralis (Gowers). These fibers occupy the antero-lateral margin of the cord, being more scattered than those of the dorsal tract of Flechsig. The ventral position of the tract is plain in figures 4, 5 and 6 for the region of the medulla. In the pons the

ventral fibers shift to their more dorsal position in the lateral margin of the brachium conjunctivum, as seen in figure 7. Throughout the cephalic half of the third block and a part of the fourth block –that is, in the lobus anterior—the fibers of the fasciculus spino-cerebellaris ventralis are distributed throughout the folia of the vermis, as seen in figure 7.

It has thus been made clear that the fibers from the legs and lower body wall pass to the vermis of the cerebellum and are distributed throughout the vermis, with the exception of the most anterior and the most posterior folia. A part of these fibers pass through the dorsal cerebellar tract and the inferior cerebellar peduncle to the caudal half of the vermis, while the fibers of the ventral tract pass through the superior cerebellar peduncle to the cephalic half of the vermis.

In the second dog the lesion was in the sixth segment and sections at the level of the operation showed that the entire dorsal cerebellar tract, and a part, if not all, of the ventral tract, were cut. Both above and below the lesion, degenerated fibers were very numerous all through the ventral half of the white columns and along the dorso-lateral margins. There were a few scattered degenerated fibers in the fasciculi gracilis and cuneatus. Above the lesion the degeneration was nearly identical with that found in the first dog. The degeneration of the cerebellar tracts was again double, though the lesion was confined to one side. There were fewer degenerated fibers on the un-operated side than in the first dog. In the distribution of the degenerated fibers in the cerebellum there was a little farther extension of the fibers into the lateral hemispheres. In the third dog the results were practically the same as in the other two. There was the same bilateral degeneration, less extensive on the un-operated side; the distribution of the fibers in the cerebellum also showed a slightly greater extension into the lateral hemispheres than is shown in the figures from the first dog.

CONCLUSIONS

1. The only symptoms caused by a lesion of the spino-cerebellar tracts in the dog are those referable to a loss of muscle sense and tone. The symptoms were bilateral in all three experiments and there was almost complete recovery in three weeks.

2. These symptoms, in a lesion of the tracts at the level of the sixth thoracic spinal nerve root, are confined to the hind legs and possibly the lower portions of the trunk.

3. The fasciculus spino-cerebellaris dorsalis, so far as its distribution in the cerebellar cortex is concerned, is confined to the caudal half of the vermis, and to the medial portion of the lateral hemispheres.

4. The distribution of fasciculus spino-cerebellaris ventralis in the cerebellar cortex is confined to the cephalic half of the vermis.

5. There is no definite cerebellar center for association regarding the hind legs.

6. The cerebellar tracts are represented by crossed, as well as by direct, fibers in the dog.

My very earnest thanks are due to Dr. Florence R. Sabin, whose coöperation and help alone have made this paper possible; and I also wish to express my thanks and appreciation to Drs. Emil Goetsch, Jacobson, and Hunnicutt, of the Johns Hopkins Hospital, for their invaluable help in operating on the dogs used.

LITERATURE CITED

Auerbach, L. 1890 Zur Anatomie der Vorderseitenstrangreste. Arch. f. path. Anat. und Phys. und f. klin. Medicin., Bd. 121.

Bing, Robert 1906 Experimentelles zur Physiologie der Tractus spino-cerebellares. Arch. f. Anat. u. Phys. Abth.

Bolk, D. 1905, 1907 Das Cerebellum der Säugetiere. Eine vergleichend anatomische Untersuchung. Erster und Zweiter Teil. Petrus Camper., Bd. 3. Dritter Teil., Bd. 4.

Bruce, A. Ninian 1910 The tract of Gowers. Quart. Journ. Exp. Phys., vol. 3.

Collier and Buzzard 1903 The degenerations resulting from lesions of posterior nerve roots and from transverse lesions of the spinal cord in man. A study of twenty cases. Brain, vol. 26.

LUNA 1906 Localizzazioni cerebellari: Contributo sperimentale anatomofisiologico. Ricerche fatte nel Lab. di Anatomia della R. Univ. di Roma, tom. 13.

1908 Einige Beobachtungen über die Lokalisationen des Kleinhirns. Anat. Anz., Bd. 32.

MACNALTY AND HORSLEY 1909 On the cervical spino-bulbar and spino-cerebellar tracts and on the question of topographical representation in the cerebellum. Brain, vol. 32.

MOTT, F. A. 1892 Ascending degenerations resulting from lesions of the spinal cord in monkeys. Brain, vol. 15.

1895 Experimental enquiry upon the afferent tracts of the central nervous system of the monkey. Brain, vol. 18.

PELLIZZI, G. B. 1895 Sur les dégénérescences secondaries, dans le système nerveux central, à la suite de lesions de la moelle et de la section de racines spinales. Contribution à l'anatomie et à la physiologie des voies cérébelleuses. Archives Ital. de Biologie, tom. 24.

VAN GEHUCHTEN, A. 1904 Le corps restiforme et les connexions bulbo-cérébelleuses. Le Névraxe, tom. 6.

VAN RYNBERK, G. 1904 Tentativi di localizzazioni funzionali nel cervelletto. Archivio di Fisiologia, tom. 1.

1908 Das Lokalisationsproblem in Kleinhirn. Ergeb. d. Phys., Bd. 7.

PLATE 1

EXPLANATION OF FIGURE

1 Dorsal view of the medulla and the cerebellum of a dog, to show the blocks into which the cerebellum was cut. The well marked groove between the lobus anterior and the lobulus simplex falls in the third block. The first and second blocks contain the lobulus medianus posterior; the third block includes lobulus simplex and a part of the lobus anterior, while the fourth block includes the rest of the lobus anterior.

2 Section of the upper cervical cord of Dog 1, to show the degeneration above the lesion. × 8. The outlines of all the sections were made with an Edinger apparatus and the degenerated fibers filled in free hand. The right side of the sections is the right side of the animal.

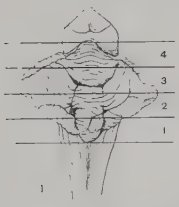
3 Section of the medulla and cerebellum of Dog 1, taken through the cephalic part of the first block as shown in figure 1. × 8. It shows degenerated fibers on both sides, in the corpus restiforme and in the fasciculus spino-cerebellaris ventralis in the medulla. In the cerebellum it shows degenerated fibers in one folium of the middle region of the lobulus medianus posterior.

4 Section through the medulla and cerebellum of Dog 1, taken through the caudal part of the second block. × 8. It shows the separation of the fibers of the corpus restiforme from those of the fasciculus spino-cerebellaris ventralis (Gowers).

5 Section through the medulla and cerebellum of Dog 1, taken through the cephalic end of the second block. × 8. It shows degenerated fibers of the left fasciculus spino-cerebellaris dorsalis, entering the lobulus medianus posterior of the vermis.

6 Section through the medulla and cerebellum of Dog 1, taken through the caudal end of the third block. × 8. The section is so near the line shown on figure 1 that the section of the pons is incomplete; it shows the lobulus simplex.

7 Section through the pons and cerebellum of Dog 1, taken through the cephalic end of the third block. × 8. It is above the level at which the corpus restiforme enters the cerebellum and shows the fasciculus spino-cerebellaris ventralis in the edge of the brachium conjunctivum and the fibers of the same tract in the lobus anterior of the vermis.

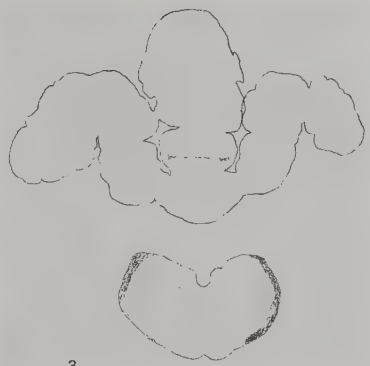


1

2

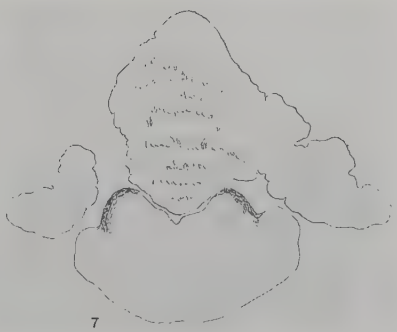
4

5



3

6



7

SOME NEW RECEPTACLES FOR CADAVERS AND GROSS PREPARATIONS

RALPH EDWARD SHELDON

From the Anatomical Laboratories, the School of Medicine, University of Pittsburgh

EIGHT FIGURES

RECEPTACLES FOR CADAVERS

Institutions which do not possess cold storage facilities usually keep cadavers in tanks of various kinds. These may be of metal (galvanized iron), wood with a metal lining (lead, zinc or copper), or of concrete. The fluids used are such, however, that the galvanized tank rusts through in a comparatively short time, since in most cases it cannot be protected by paint, on account of the solvent power of the alcohol and carbolic acid used. The stock is usually thin, necessitating the use of angle irons or planks in order to prevent bulging. Also on account of this weakness, if the tank contains material, it may not be moved without injury to the bottom. The lined tanks, which must be soldered at the corners, likewise eventually leak, the solution then making its way into the spaces between the lining metal and the wooden support. The lining is rarely smooth, considerable dirt accumulating, therefore, in cracks and uneven places. On account of these factors, the tank becomes foul and undesirable in a well-kept laboratory. Concrete tanks properly built and lined with cement are satisfactory, excepting that they cannot be moved and that they take up a large amount of space.

After some experience with various types of receptacles, the tank described below was designed for the Anatomical Laboratories at Pittsburgh. It has now been in use for more than a year and has given perfect satisfaction. Essentially it consists of a box built of two-inch cypress with half-inch bolts running in the wood, horizontally through the bottom and vertically in the sides and ends. The individual pieces of wood are fitted together as shown in figure 1. By this method of construction a solid and exceedingly strong box is obtained. If any leakage occurs through excessive drying, it is necessary only to tighten the nuts on the bolts. The case is further strengthened by two angle irons (*d*, figs. 2 and 4) running lengthwise along the bottom at the corners. Through these pass the horizontal bolts (*a*, figs. 2, 3 and 4), also the vertical bolts (*c*, fig. 3). The ends of

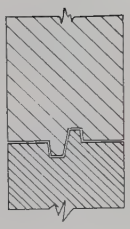
all other bolts pass through bars of iron one-half inch by two inches; *e*, figure 2, for the horizontal bolts, *b*, figure 2; *f*, *g* and *h*, figures 2 and 3, for the vertical bolts *c*. These strengthen the case and prevent heads and nuts from being drawn into the wood through its swelling or the tightening of the nuts. Two strips of oak one inch by six inches (*i*, figs. 2 and 3) are placed lengthwise under the tank in order to permit the floor underneath to be cleaned. These are removable so that they may be eventually replaced. It will be noted that they do not come in contact at any point with the wood forming the floor of the tank, thus preventing its rotting. The top is hinged and consists of one-inch cypress, properly battened. A gate valve for emptying the tank is desirable. Hot oil should be applied to the raw wood unless it is to be painted.

While this tank is moderately heavy, it can be easily moved when empty, or even when partially full, as it is not injured by the use of levers. Even when full of solution there is no bulging of the sides. Since all parts are tightly fitted, it may be used for the storage of cadavers in alcohol fumes. In fact, such a tank has been thus used here for nearly a year, the material keeping perfectly. Such a receptacle, six feet, six inches long by two feet, ten inches wide and two feet, eight inches high, inside measurements, will hold fifteen cadavers of average size. It is practically indestructible and should last indefinitely. The first cost is sixty dollars.

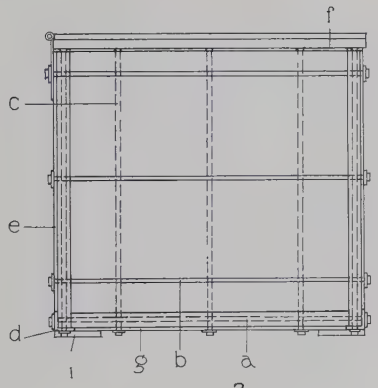
RECEPTACLES FOR GROSS PREPARATIONS

Large gross preparations, particularly dissections, are ordinarily kept in tanks similar in structure to those mentioned for the storage of cadavers, although often smaller in size. Such tanks are subject to the same disadvantages as when used for cadavers. In addition, they are usually unsightly in appearance and therefore undesirable in laboratories and museums. Glass jars of sufficient size for large human preparations are very expensive and easily broken; earthen crocks are likewise very fragile. It is not feasible, moreover, to use either of these for large preparations, such as longitudinal sections. The receptacles described below are designed to serve as museum cases for large specimens and as storage receptacles for material which it is desired to have constantly available for students or members of the instructing staff.

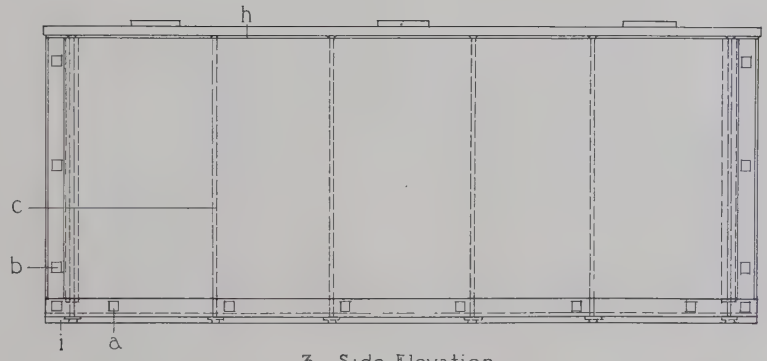
These cases are constructed on essentially the same plan as the cadaver tanks. Lighter material, however, may be used in their construction, since the cubic contents is considerably less. The cypress used is somewhat thinner, the bolts are three-eighths inch instead of one-half inch, while the angle irons and iron bars are omitted entirely, washers being used instead. The tank proper (*j*, fig. 6) is placed on a removable base (*k*, fig. 6) in order to make it of the proper height for use. The top, which is largely of glass, is sloped forward in order to give a good view of the contents when the case



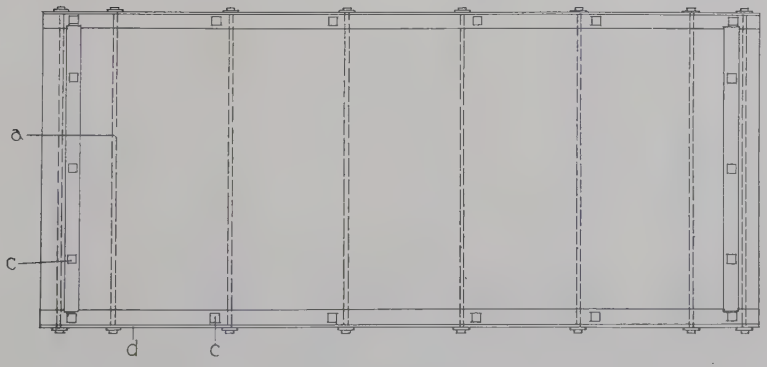
1



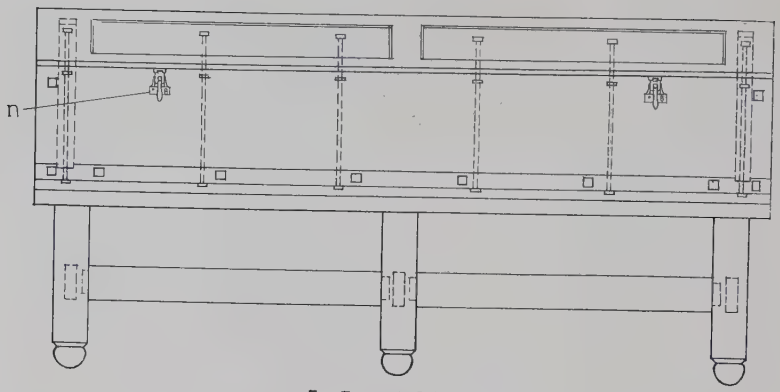
2.



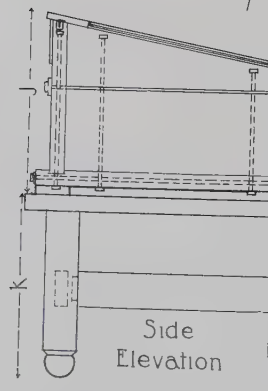
3 Side Elevation.



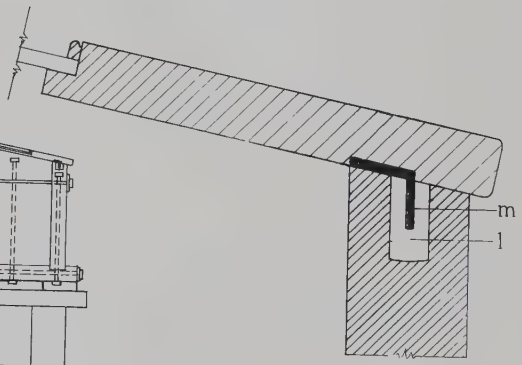
4 Plan of base



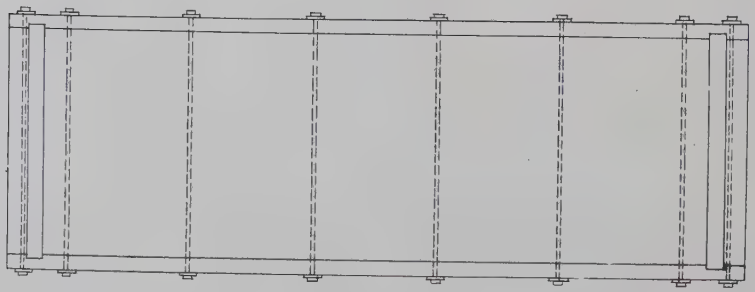
5. Front Elevation



6.



7



8. Plan of base.

is used for museum preparations. The groove (*l*, fig. 7) runs entirely around the case; into it projects one arm of an angle iron (*m*). When the groove is filled with cotton the case is made practically air-tight so that there is almost no evaporation where it is desired to keep material in alcohol fumes. The catches used are Corbin tool box locks number 1217 (*n*, fig. 5). By the use of catches of this type, it is possible always to draw the cover tight and likewise lock the case if desired.

At Pittsburgh, we have placed cases of this type in the dissecting-room, where we have found them very useful for longitudinal sections of cadavers and for large gross dissections kept as museum preparations. One case is used for the best dissections made each year by the class, which are thus available the succeeding year as demonstration preparations. Cases of this type, two feet two and a half inches wide, eight feet long, one foot high in front, one foot, six inches in the rear, inside measurements, with a base, may be secured finished for forty-two dollars each.

Acknowledgments should be made to Mr. E. B. Lee, architect, Pittsburgh, for the preliminary sketches. It should also be stated that the idea of using bolts in the manner indicated above was first suggested to me by some small wooden cases which I saw some years ago in the Anatomical Laboratories at the University of Pennsylvania.

INCREASE IN PRICE OF JOURNALS

In order to extend and improve the journals published by The Wistar Institute, a Finance Committee, consisting of editors representing each journal, was appointed on December 30th, 1913, to consider the methods of accomplishing this object. The sudden outbreak of European misfortunes interfered seriously with the plans of this committee. It was finally decided, at a meeting held December 28th, 1914, in St. Louis, Mo., that for the present an increase in the price of these periodicals would not be unfavorably received, and that this increase would meet the needs of the journals until some more favorable provision could be made.

This increase brings the price of these journals up to an amount more nearly equal to the cost of similar European publications and is in no sense an excessive charge.

The journals affected are as follows:

THE AMERICAN JOURNAL OF ANATOMY, beginning with Vol. 18, price per volume, $7.50; foreign, $8.00.

THE ANATOMICAL RECORD, beginning with Vol. 9, price per volume, $5.00; foreign, $5.50.

THE JOURNAL OF COMPARATIVE NEUROLOGY, beginning with Vol. 25, price per volume, $7.50; foreign, $8.00.

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY

36th Street and Woodland Avenue

Philadelphia, Pa.

VASCULARIZATION PHENOMENA IN FRAGMENTS OF EMBRYONIC BODIES COMPLETELY ISOLATED FROM YOLK-SAC BLASTODERM

FRANKLIN PEARCE REAGAN

Department of Comparative Anatomy, Princeton University

TEN FIGURES

Recent experimental work points to the possibility of a final solution of the problem of the origin of intra-embryonic vascular endothelium. Methods of procedure affording results to which there can be no doubt of interpretation are especially desirable.

So far there have been developed two methods by which yolk-sac 'Angioblast' may be kept out of communication with intra-embryonic vessels: mechanical separation of the vessels of these two regions, and exposure of the developing embryo to anesthetics. The former method was employed to the extent of partial separation by Gräper,[1] Hahn,[2] and Miller and McWhorter.[3] These observers have obtained endothelium on both sides of chick embryos in which one side was severed from extra-embryonic blastoderm. The second method has been perfected by Stockard[4] who has, in cases of arrested development, been able to secure intra-embryonic endothelium quite independent of that in the yolk-sac.

The intra-embryonic vessels in the experiments of Miller and McWhorter were in part non-continuous and somewhat diminutive in size; this is perhaps due to the fact that heart-pressure is necessary for the normal growth of endothelium even after the latter has been established. The work of these two ob-

[1] Gräper, L., Archiv für Entw. mech., Bd. 24, 1907.

[2] Hahn, H., Archiv für Entw. mech., Bd. 27, 1909.

[3] Miller, A. M., and McWhorter, J. E., Anat. Rec., vol. 8, p. 91, 1914.

[4] Stockard, C. R., Proc. Am. Assn. Anatomists, Anat. Rec., vol. 9, **no. 1,** 1915.

servers was submitted as proof of the local origin of intra-embryonic endothelium; previous to and following its publication, this work was objected to quite vigorously on the following grounds: the incision may not have been made sufficiently early or sufficiently close to the embryonic body; vessels may have grown into the injured side from the unoperated side or from either end. To the latter objection Miller has replied that this unusual growth would require a permeation of such solid structures as notochord and neural tube. A later examination of Miller's material by Bremer, as well as Miller's own careful reconstructions, failed to reveal such ingrowths.

While the work of Miller and McWhorter seems in itself to be quite conclusive, a confirmation of their results by more rigorous methods of experimentation may not be superfluous. A most feasible method of procedure seems to be that of complete separation of an embryonic body, or a portion thereof, from the extra-embryonic blastoderm prior to a possible 'invasion' by the so-called yolk-sac 'Angioblast.' If in such a meroplast there should develop legitimate vascular cavities possessed of good endothelium it is confessedly futile to argue further for the necessity of 'Angioblastic' origin of intra-embryonic endothelium.

The following experiments meet, I believe, the objections urged against the work of Miller and McWhorter, supplementing at the same time the work of Stockard.

About forty chick embryos corresponding to stages 4, 5, 6 and 7 of Kiebel's Normentafel (Zweites Heft) constitute so far the material studied. The operations consist in varying degrees of separation of the projecting head from the remainder of the embryonic body and the blastoderm.

One experiment which I shall designate as Type I consisted in the following incisions (fig. 1): longitudinal incisions lateral to, but close to the projecting head on each side, extending from points slightly posterior to the anterior intestinal portal to the opaque area anteriorly; a transverse incision through the embryonic body just posterior to the anterior intestinal portal. Following this the blastoderm was entirely removed except the

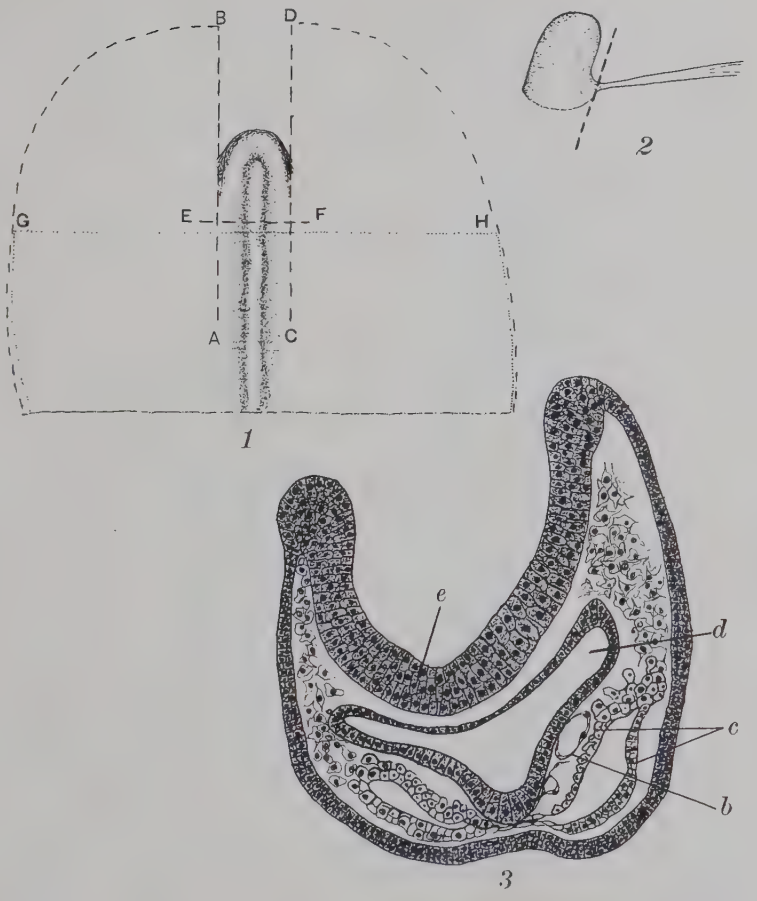


Fig. 1 Diagram to illustrate the blastodermal incisions in experiments of Types I and II. Broken lines represent the incisions in Type I. Dotted line *G* to *H* indicates the transverse incision in Type II in which incision *E* to *F* is omitted.

Fig. 2 Lateral view of the head-fragment of Type I pushed back to be severed from the proamnion at point where a broken line intersects.

Fig. 3 Section through the anterior portion of the forebrain of a head-meroplast, showing unusual head-coelom. Total incubation thirty-two hours. Operation at the time of the first intersomitic groove (× 160). Experiment, Type I, no. 19; *b*, anlage of ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

small strip of proamnion which suspended the head-fragment from the opaque area anteriorly. (In this way it was possible to section later the blastoderm and determine the status of vascular development in the extra-embryonic area and *to know certainly that the embryo had not yet been vascularized*). The head-fragment was then pulled backward and turned so that the proamnion could be snipped off close to the head-tissue proper (fig. 2), leaving a free meroplast which would sink to the bottom of the sub-germinal cavity. The egg was then sealed and incubated further.

Although many other methods were utilized, it will suffice for the present work to describe one more experiment—Type II. Longitudinal and transverse incisions were made as in Type I, except that the transverse incision extends entirely across the blastoderm. The blastoderm was removed to be sectioned while the head-fragment was left connected anteriorly with the opaque area by the small strip of proamnion. Thus the head-fold rested on a double membrane of ectoderm and entoderm which was also excised laterally but not anteriorly from the opaque area.

Complete separation of the posterior region proved to be quite unsatisfactory owing to the circumstance that the relief of normal surface tension induces abnormal conditions in the embryonic body. In the projecting head-region surface tension evidently does not enter so extensively into the mechanics of development. Furthermore a relatively small amount of injury in the head region serves to isolate completely an embryonic fragment in which development proceeds in a surprisingly normal manner.

In order to preclude the possibility of drawing conclusions from tissue which had already been 'invaded' by 'Angioblast,' the remainder of the blastoderm which had been removed at the time of operation was sectioned. The region of the embryonic body which would have first been vascularized was contained in the axial portion of such blastoderms. Before each incision the instruments were sterilized. These precautions together with that of complete isolation should render the

procedure sufficiently rigorous to satisfy all reasonable demands of experimental proof.

The tissue was fixed in a picro-acetic mixture; sections were stained in a modification of Mann's methyl blue-eosin stain,[5]

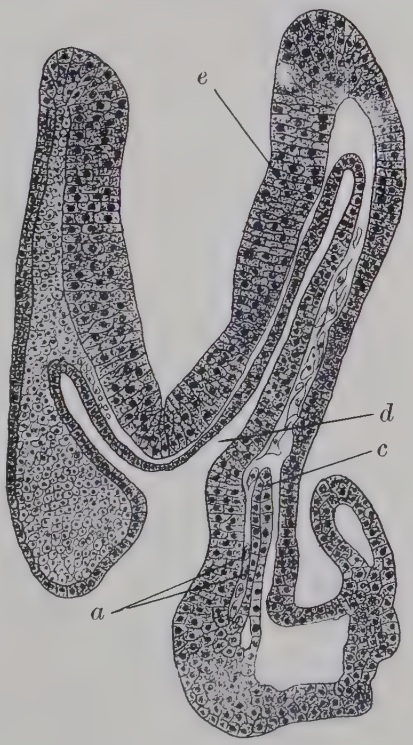


Fig. 4 Section through the forebrain of a head-meroplast, showing early stages in the formation of vasofactive cells. Total incubation, twenty-nine hours: Operation previous to the formation of the first intersomitic groove (× 200). Experiment, Type I, no. 24; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

which proved especially valuable in the differentiation of endothelium.

[5] Reagan, F. P., Anat. Rec., vol. 8, no. 7, 1914.

When the head-fragments as above described had been incubated for a total period of from thirty to forty-eight hours and then sectioned, they were found to *possess blood vessels in varying degrees of development.* In general it may be said that regardless of the amount of incubation beyond a total period of thirty-three hours, differentiation never proceeded beyond the normal stages of differentiation at that age. After forty-eight hours some signs of degeneration made their appearance. It seems that the embryonic meroplast possesses an inherent capacity for differentiation which tides it over to the time when heart-pulsations would normally provide a means of tissue respiration. While differentiation proceeds always at the same rate and to the same extent, growth varies greatly. Meroplasts equally differentiated may vary greatly in size.

Practically the only unusual condition met with in these head-fragments is the presence of a head coelom (fig. 3), the origin, fate and significance of which will be considered later.

Between the base of the coelomic pouch and the pharyngeal entoderm rounded or cuboidal cells become proliferated. Their point of origin is in most instances between the base of the coelomic pouch and the pharyngeal entoderm (figs. 4, 5 and 7), where it is difficult to determine which of these two epithelia is of primary importance in such cell proliferation. Cells of this sort are undoubtedly proliferated to a certain extent by the pharyngeal wall and also by mesothelium. In neither of these latter cases do the cells originate by foldings and constrictions of cell aggregates, but singly or in a linear proliferation. This same region is found, in somewhat later stages (fig. 4) to be occupied by irregular stellate cells resembling mesenchyme cells. Their processes fuse forming a parenchyma-like complex which merges dorsally into the true interstitial mesenchyme.

Simultaneously with the accumulation of a plasma-like fluid which may be detected in this parenchyma by sections of its coagulum, the loose structure becomes transformed into a longitudinal tube of endothelium (figs. 6 and 7). The tubes thus formed, though far anterior to the heart-region, may simulate heart-formation in a remarkable manner. The coelomic pouches may meet to

possess a continuous cavity, a condition approached in figure 6. Since the pharynx in this region is already tubular it does not become constricted in this process, the endothelial tubes meeting ventral to it. There is no reason to believe (though such an inference is possible) that the endothelial tubes thus formed are new or abnormal formations; they occupy the position of normal ventral aortae.

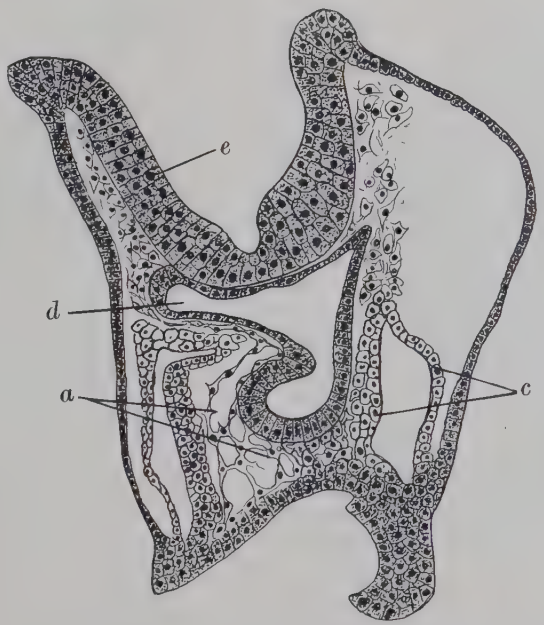


Fig. 5 Section through the forebrain of a head-meroplast showing a loose parenchyma in a position occupied by the isolated vasofactive cells of figure 4. Total incubation thirty hours. Operation at the time of the first intersomitic groove (× 150). Experiment, Type I, no. 18; *a*, prevascular mesenchyme; *c*, coelom; *d*, pharynx; *e*, forebrain.

The conditions so far described are found in embryonic fragments of Type I which were incubated not more than a total period of thirty-three hours. It will be well to consider some of the conditions found in a meroplast of Type II incubated for

7 6

Fig. 6 Section through the forebrain of a head-meroplast showing anlagen of the ventral aortae as discrete vessels. The longitudinal incision on the right side of the head was relatively close to the neural fold. The cut edges of the pharyngeal entoderm have been pulled apart, the ventral tissues having swung to the left. Total incubation thirty-two hours. Operation at the time of the second intersomitic groove (× 240). Experiment, Type I, no. 31; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

Fig. 7 Section through the forebrain of a head-meroplast showing a well-defined ventral aorta. The right longitudinal incision was close to the neural fold. None of the excised head has regenerated; the mesenchyme is very compact near the cut surface the cells of which are somewhat epithelial. Total incubation thirty-three hours. Operation at the time of the first intersomitic groove (× 175). Type I, no. 17; *b*, ventral aorta; *c*, coelom; *d*, pharynx; *e*, forebrain.

a total period of forty-eight hours (figs. 8, 9 and 10). No attempt will be made at present to set forth the processes which intervene between the stages of thirty-two and forty-eight hours.

The proamnion (fig. 8), a region normally devoid of mesoderm, contains a rounded pouch which is continuous anteriorly with the extra-embryonic coelom. The cut edges of the ectodermal and entodermal layers of the proamnion have fused forming a blind sac around the enclosed coelomic pouch; likewise the cut

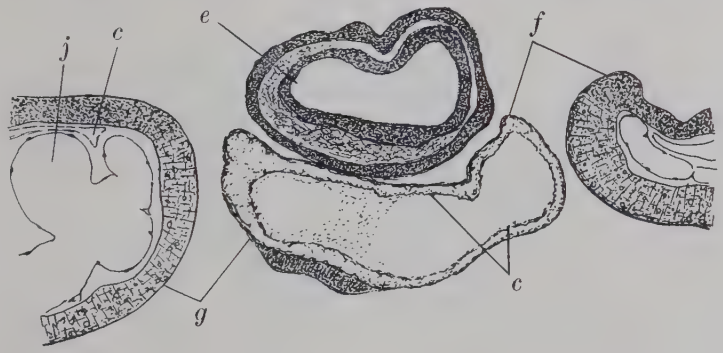


Fig. 8 Section through the forebrain of a head-meroplast showing a proamniotic sac containing a pouch of coelomic mesothelium. On the ventral side of the sac is a peculiar proliferation of entodermal cells very constantly appearing in experiments of this type, generally more symmetrically situated. Total incubation, forty-eight hours. Operation at the time of the second intersomitic groove (× 55). Experiment, Type II, no. 3 *c*, coelom; *e*, forebrain; *f*, ectoderm; *g*, entoderm; *j*, extra-embryonic vessels.

edges of the once continuous blastoderm have fused. On the ventral surface of the proamniotic sac will be noticed an entodermal thickening, in appearance not unlike an inverted neural groove. This structure is quite constant in experiments of this type. I would interpret it as a cell-complex representing potentially the floor of the fore-gut in case of normal infolding.

The transverse incision in this experiment was made some distance behind the site of the anterior intestinal portal, so that there projected behind the posterior extent of the proamniotic

sac a portion of the embryonic body bounded dorsally by ectoderm and ventrally by entoderm (fig. 9). The cut edges produced by the longitudinal incision have fused, the point of fusion being indicated by the apex of the projection on the left side in figure 9. In this figure we have a photograph of a 'projecting' head; the section passes through the anterior part of the midbrain region, presenting a rather puzzling condition in that it is entirely devoid of fore-gut. Two large dorsal aortae are present; the larger one is located on the side containing the greater amount of mesenchyme; correlated also with the fact that the

Fig. 9 Photograph of a section through the midbrain of the same meroplast as in figure 8, showing well developed dorsal aortae and the absence of a tubular pharynx in a tubular head. Fusion of entoderm and ectoderm at points indicated by x (× 120). f, ectoderm; g, entoderm; h, dorsal aorta; i, midbrain.

incision on this side was made at a greater distance from the median line. Both aortae are bounded by distinct and unmistakable endothelium. In the aortic cavities blood plasma has coagulated. No coelomic pouches are present in this section. Heart-formation has not taken place in this particular experimental case.

The phenomena under consideration are not to be regarded as regenerative changes. The head does not regenerate the yolk-sac, neither does the yolk-sac regenerate the head. In figures 6 and 7 it will even be seen that portions of the head itself were not regenerated. There is a genesis of the first order in

case of the development of endothelium. While it is not always possible to be sure of the extent to which experimental conditions portray a truly normal process, the results here presented, together with those produced by Stockard seem to afford positive evidence in favor of the local origin of blood vessels.

It is of interest to note the statement of Bremer (Am. Jour. Anat., vol. 16, no. 4, p. 463) that mesothelial anlagen "might arise under abnormal conditions in positions where they are normally absent." The isolated cells proliferated from this

Fig. 10 Photograph of one of the first available sections of the blastoderm behind the incision *G* to *H* of Experiment Type II, no. 3, showing the freedom of the pellucid area from endothelium (× 160).

unusual mesothelium are, according to my observation, not comparable to the gross infoldings of cell-aggregates described by Bremer, though they seem to be vasofactive in nature. To designate certain of these proliferated cells as mesothelium would be as uncalled for as to designate others of undoubted entodermal origin as pharynx. While I do not question the ability of mesothelium to proliferate pre-vascular mesoderm (indistinguishable from mesenchyme), I do wish to question the justice with which Bremer would accredit mesothelial tissue with the production of the entire vascular tissue. An assignable reason for conferring this distinction on mesothelium might be the desire to maintain for endothelium a monogenetic origin—the first requisite to the specificity of a tissue.

The fact that endothelium exists in the sauropsidan yolk-

sac prior to the establishment of a coelom cannot be satisfactorily explained by a hypothetical "premesothelial stage of mesoderm" (Bremer, loc. cit., p. 463). It has been shown that 'Angioblast,' so far from being a daughter-tissue of premesothelial mesoderm, is really a parent-tissue of the latter; in isolated blood islands Rückert[6] has shown that cell groups proliferated from this early vascular tissue cleave to form slit-like cavities which unite later with other similarly formed cavities to contribute to the extra-embryonic coelom. Of logical necessity it follows that mesothelium and 'Angioblast' (in the sense of His) must have come from a common cell-complex.

Should we ever be so fortunate as to find the ultimate font and source of all vascular tissue there would be no objection to its designation as 'Angioblast,' so long as we bear in mind the original implications of the Angioblast Theory; the essentials of this theory have been outlined by Minot (Human Embryology, Keibel and Mall, vol. 2, pp. 498–99) as follows:

> Comparative embryology teaches that the first bloodvessels appear on the yolk-sac collectively and at one time. They form a unit anlage which we call angioblast according to the suggestion of His. * * * The angioblast probably maintains its complete independence throughout life. In other words it is probable that the endothelium of the blood vessels (and of lymph vessels) and the blood cells at every age are direct descendants of the primitive angioblast.

The fact that allantoic vessels may appear *prior to* those in the yolk-sac in early human development (Bremer, ibid.) is probably an expression of the tendency towards that sequence of local origin which is correlated with the functioning of the part vascularized. A different sequence is found in animals where the allantois functions relatively later.

When it is stated that endothelium differentiates from an 'indifferent' mesenchyme the specificity or non-specificity of the immediate source of the pre-vascular mesenchyme is not

[6] Rückert, J. Entwickelung der extra-embryonalen Gefässe der Vögel. Handbuch der Vergl. u. exp. Entw-lehre, Bd. I, T. 1, 1906. Ueber die Abstammung der bluthaltigen Gefässanlagen beim Huhn, und über die Entstehung des Randsinus beim Huhn und bei Torpedo, Sitzungsber. der Bay. Akad. Wiss., 1903.

brought into question; the statement merely means that in their earliest stages such cells are morphologically indistinguishable from other mesenchyme cells which may or may not be capable of like development. Whether each vasofactive cell behaves as it does in accordance with a definite teleology is at present entirely beside the question.

Like considerations apply to the study of 'indifferent' capillary plexuses, some of the meshes of which persist while others degenerate. That one should observe this process from time to time, and from the mere fact that the process takes place, be able to determine the extent to which the changes are due to heredity or mechanical influence is to be accepted with some caution; cell organization is excluded with great difficulty. In a regenerating jugular vein Clark[7] believes he has a case in which heredity plays no part. It may be stated that authorities on regeneration do not usually exclude considerations of heredity from the conclusions at which they arrive. Certain it is that the development of the vascular system furnishes an unproductive field for the solution of the problems of preformation and epigenesis.

In conclusion it is well to consider the following recently established facts which should share in defining our morphological interpretations. The yolk-sac is not necessarily the site of formation of the earliest blood vessels. Intra-embryonic vessels develop *in situ* when communication of extra-embryonic vessels with intra-embryonic tissues is prevented by chemical or mechanical means.

[7] Clark, E. R., Proc. Am. Assn. Anatomists, Anat. Rec., vol. 9, no. 1, 1915.

MEMOIRS

The publication of this series of Anatomical Monographs has been undertaken with the purpose of presenting the results of original investigation in anatomy which are too extensive for incorporation in the already over-crowded current periodicals.

No. 1. The Anatomy and Development of the Systemic Lymphatic Vessels in the Domestic Cat, by George S. Huntington, Professor of Anatomy, Columbia University, New York City. This monograph states in a few pages the various theories held in regard to lymphatic development in general and then proceeds to present the result of six years' careful investigation on mammalian lymphatic development. Part I deals with the development of the systemic lymphatic vessels in their relation to the blood vascular system. Part II deals with the development of the preazygos and azygos segments of the thoracic duct. This memoir contains 175 pages of text, 8 text figures (two in color), 254 photomicrographs and 21 colored plates. Sent post paid to any country for $4.00

No. 2. Contribution to the Study of the Hypophysis Cerebri with Especial Reference to its Comparative Histology, by Frederick Tilney, Associate in Anatomy, Columbia University, New York City. Part I contains a historical review of the literature. Part II deals with the comparative histology of the pituitary gland and gives a report of six hypophysectomies performed upon cats. This memoir contains 72 pages of text, 2 text figures, 60 photomicrographs and plates. Sent post paid to any country for $1.50.

No. 3. Early Stages of Vasculogenesis in the Cat (Felis Domestica) with Especial Reference to the Mesenchymal Origin of Endothelium, by H. Von W. Schulte, Department of Anatomy, Columbia University, New York City. This memoir contains 90 pages of text and 33 figures of which 14 are in colors. Sent postpaid to any country for $1.50.

Orders should be addressed and checks made payable to

THE WISTAR INSTITUTE OF ANATOMY AND BIOLOGY
Philadelphia, Pa.